CHAPTER ONE

1 OVERVIEW

1.1 INTRODUCTION

Diet, dietary supplements and medications can be used to influence growth and health in animals. Being able to control the rate and time these supplements/ compounds are automatically delivered can decrease the amount required and even out the delivery, which can decrease overall delivery cost. Today, many controlled release drug delivery methods are possible. For instance, desired drug release can be provided using rate-controlling membranes or polymer-based delivery systems (Langer, 2000; Ulbricht, 2006). There has been extensive research to design appropriately functionalised polymeric systems and recent advances in polymer science and drug carrier technologies have helped develop novel drug carriers (Langer and Peppas, 2003). In this thesis the term *drug* has been used to cover any compound, including medication, nutrients, and other active ingredients.

Controlled release drug delivery and its applications have attracted much attention over the last decade. The criteria for choosing materials to act as the foundation for a system are challenging. These materials used must be safe and non-toxic, possess acceptable biocompatibility and not cause excessive immune responses. They must also possess appropriate mechanical properties and be suitable for manufacturing techniques that generate high surface area porous structures.

Materials that combine such characteristics can be divided in two main categories: natural polymers including alginate, collagen, dextran, chitosan and gelatin and synthetic polymers including polyesters, polyaminoamides, polyacrylates and their copolymers and blends (Hoffman, 2002).

A wide range of polymers can be used to fabricate carriers for controlled release. The first question when faced with the challenge of encapsulating a substance should be "what is the final application of the product?" For example, if the final target is an encapsulated pesticide, one must first choose polymers that are stable and non-erodible. For encapsulating therapeutic drugs used in human or animal, the choice of polymers becomes more restricted to bio-compatible with tissue and non-toxic. After selecting the polymer system, the next step is to select an appropriate encapsulation method.

Microencapsulation is a process in which small particles or droplets are entrapped by a coating to give the particles with many useful properties. Microcapsules have been widely studied for their potential applications in the pharmaceutical, agricultural, and chemical industries. They have several advantages as drug carriers including large specific interfacial area, high selectivity, maximal loading ratio, and most importantly stability. However, the number of polymers that can be used to form microcapsules is limited. Different encapsulation methods result in either a microcapsule or microsphere. A microcapsule is a system that contains a well-defined core and a well-defined shell (Mathiowitz *et al.*, 1999). The core can be solid, liquid, or gas and the shell is made of a continuous, porous, or nonporous, polymeric phase. On the other hand, a microsphere is a structure made of a continuous phase of one or more miscible polymers in which particulate drug are dispersed.

In recent years, several research groups have been developing responsive systems that would more closely emulate the normal physiological process in which physiological needs influences the amount of drug released. Added control over the release profile can be obtained by using core-shell microcapsule and pH-sensitive systems. The enhancements presented by such systems are discussed in this thesis.

There are many applications of controlled delivery to animals and the list of products using controlled delivery continues to grow. Ruminant animals such as cattle, sheep and goats are major sources of meat, milk, wool and leather and therefore important segments of the agricultural economy (Vandamme and Ellis, 2004). Ruminants have a four-compartment stomach composed of the rumen, reticulum, omasum and abomasums. The rumen fluid has a neutral pH within the range of about 5.5–7.0, and the abomasal fluid has an acidic pH of about 2.0–3.0. Rumen fermentation can modify and/or inactivate many pharmacologically active ingredients administered to the host animal via the oral route (Harfoot, 1978). Advances in ruminant nutrition and health demand a rumen-stable delivery system that can deliver the active ingredient post-ruminally while simultaneously meeting efficacy, safety and cost criteria. In contrast to drug delivery systems for humans, the need to have low-cost systems has hindered developing effective rumen-stable delivery systems to protect bioactives from the harsh environment in the rumen.

The objectives of this thesis are to investigate a post-ruminal targeted delivery system based on polymeric microencapsulation, which involves examining ways to develop reversible and switchable drug protection technologies using smart polymers that respond to changes of pH in the *in vivo* environment. Such protection will stop active ingredients breaking down or being absorbed in undesired sites in the body whilst allowing the drug to be absorbed at other sites. Thus, the drug can either be protected while it passes through harsh environments (in this case the rumen) or customised to maximize uptake rates at a specific absorption site (post-rumen). The primary goal of this study is to focus on developing a novel technology to protect bioactives from microbial degradation in the rumen and target their release to the abomasum. A microcapsule that is closed in the rumen and open in the abomasums, using pH as the stimulant or switching gate to change permeability of the microcapsules, is a suitable technology. One of major objectives is to develop microcapsules with a strong, thin shell and a large hollow centre. This will maximise drug-carrying capacity yet be strong enough to withstand harsh physical conditions in the rumen.

1.2 THESIS OUTLINE

- Chapter One provides briefly an introduction, overview and structure of this thesis.
- Chapter Two contains a critical evaluation of the literature, covering controlled release drug delivery, ruminant applications, biomaterials, polymeric systems, surface modification of polymer materials, encapsulation techniques, and the hypothesis for research.
- Chapter Three details the methodology, experimental materials and chemicals used in the research.
- Chapters Four and Five discuss the results and characteristics of microcapsules investigated in this thesis. Four types of produced microcapsules: polyamide, polyester, polysulfone, and polystyrene are discussed in separate sections in terms of preparation, functionalisation, and characterisation. This thesis focuses on two candidates of microcapsules (polyamide and polystyrene) to carry out more detailed studies to evaluate the release profiles of model drugs. Also it discusses the data obtained and relates it to the published information.
- Conclusions and recommendations are given in Chapter Six. Raw data are presented in the Appendix.

CHAPTER TWO

2 CRITICAL REVIEW OF THE LITERATURE

2.1 CONTROLLED RELEASE TECHNOLOGY

Drug delivery systems are based on interdisciplinary approaches and combine pharmaceutics, polymer science, bioconjugate chemistry, and molecular biology. Research and development in drug delivery systems has achieved significant progress. Since the 1980s, the pharmaceutical industry has witnessed significant research and development between the fields of polymer and material science, which has resulted in the development of novel drug delivery systems that facilitate site-specific therapy and sustained delivery (Mathiowitz *et al.*, 1999). Examples of such drug delivery systems include polymeric carriers, microspheres, micelle-forming block copolymers, liposomes and hydrogels.

With the development of more potent drugs, the drawbacks of conventional therapeutic systems have become more apparent. These include adverse effects, strongly fluctuating drug levels in the body, poor drug efficacy, and poor patient compliance. The concentration, duration, and bioavailability of the pharmaceutical agents could not be controlled. Controlled release technology was developed to circumvent these problems. Their aim is to maintain therapeutic concentrations of a drug in the body for sustained periods by releasing the agent in a predictable and controllable fashion (Figure 2.1). A new development, polymeric controlled drug

delivery, has evolved from the need for prolonged and better control of drug administration. The first polymeric devices developed for controlled drug release date back to the early 1960s (Wichterle and Lim, 1960).

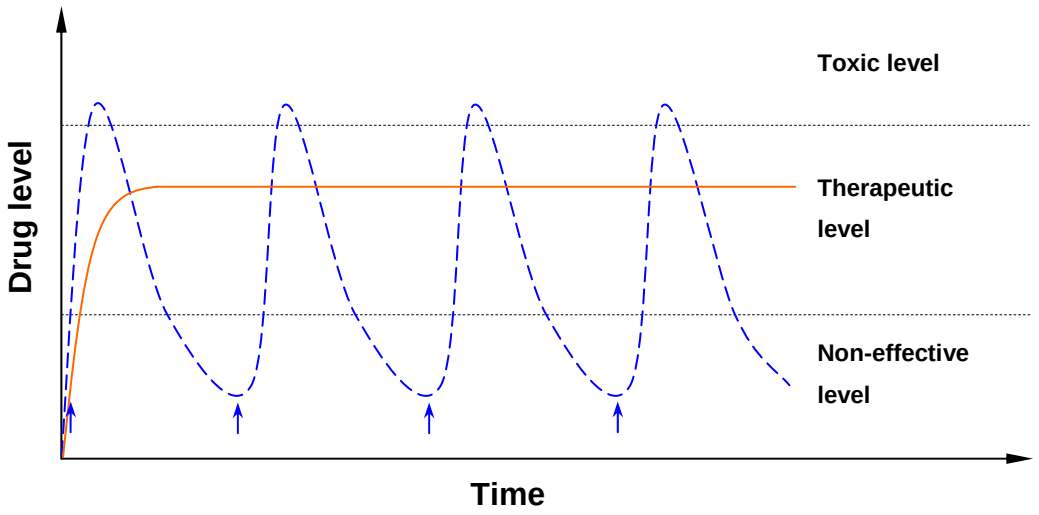

Figure 2.1 Comparison of conventional (- - -) and ideal controlled release delivery (—) systems

Improved drug delivery has the following advantages (Langer, 1998):

1. Able to continuously maintain drug levels in a therapeutically desirable range;
2. Reduces harmful side effects because the drug is delivered to a particular cell type or tissue rather than to any cell or tissue;
3. Potential to decrease the amount of drug needed, thus reducing costs;
4. Decrease the number of dosages required and possibly using less-invasive dosing, leading to improved patient compliance with the prescribed drug regime;
5. Facilitates the administration of pharmaceuticals with short *in vivo* half-lives (for example peptides and proteins).

Controlled delivery formulations can increase the efficiency of labile drugs. Once released from the dosage form, the drug must pass through several physiological barriers before reaching the action site; it must then survive metabolic and chemical attacks. Labile drugs, typically peptides, proteins, and enzymes, may lose activity due to the local tissue environment, such as the acidity in the stomach

(Allemann *et al.*, 1998). Therefore, they need to be protected in some way such as being encapsulated in a biocompatible polymer that will act as a transient mask and protect the therapeutic agent from physiological degradation until the desirable site is reached.

2.2 CONTROLLED DELIVERY IN ANIMALS

Controlled delivery to animals can be classified into two broad categories: systems for food-producing animals and those for companion animals. Food-producing animals consist primarily of cattle, sheep, swine, and poultry, together with fish and any other animal from which meat or other products such as eggs or milk are obtained. Companion animals are those considered as pets, primarily dogs and cats, but can include horses, birds, lizards, rabbits (Ahmed & Kasraian, 2002). This section of the thesis discusses applications of controlled drug delivery to ruminants.

There are many applications of controlled delivery to animals and the list of products using controlled delivery continues to grow (Winzenburg *et al.*, 2004). The animal health market in the United States was nearly $3 billion in 1997 (Table 2.1) (Rathbone *et al.*, 1999). By 2003, the United States market was worth more than $7.3 billion and made up 39% of global sales in the sector (The-infoshop.com, 2009). By 2005, the world market for animal health products was US$17.4 billion (Research and Markets, 2009).

Table 2.1 Summary of U.S. animal health industry market

Therapeutic area	Sales (millions of $) in 1997
Antibacterials	551.9
Biologicals	483.2
Hormones	266.6
Insecticides and parasiticides	414.1
Internal parasiticides	540.2
Vitamins and nutritionals	134.7
Other	415.3
Total	2,806

2.2.1 Ruminant physiology

Ruminant animals such as cattle, sheep and goats, are major sources of meat, milk, wool and leather and therefore important segments of the agricultural economy. Their role in the food chain is particularly prominent because they utilize cellulose and non-protein nitrogen (which are abundant in nature) that are either not utilized or poorly utilized by other farm animals and humans. This ability is due to the ruminant's digestive system, which is a unique symbiotic relationship of the host animal with bacteria and protozoa (Harfoot, 1978). Rumen fermentation can be both beneficial by enabling the utilization of cellulose and non-protein nitrogen and detrimental by reducing the nutritive value of some carbohydrates, high-biological-value proteins and by hydrogenating unsaturated lipids. It can also modify and/or inactivate many pharmacologically active ingredients administered to the host animal via the oral route.

In contrast to drug delivery systems for humans, the need for low-cost systems has hindered development of effective rumen-stable delivery systems. Advances in ruminant nutrition and health demand a rumen-stable delivery system that can deliver the active ingredient post-ruminally while simultaneously meeting efficacy, safety and cost criteria. Vandamme and Ellis (2004) reviewed the necessary concepts, the issues, and the challenges in developing ruminal drug delivery systems; and also described patented, marketed, and principles of the design of ruminal drug delivery devices.

Ruminants have a four-compartment stomach composed of the rumen, reticulum, omasum and abomasum (Figure 2.2). The rumen is the largest compartment and metabolically the most important. The reticulum is separated from the rumen by a ridge of tissue and the omasum lies on the right hand side of the stomach and connects with the reticulum and abomasum. The latter is a tubular organ connecting the omasum with the small intestine (Harfoot, 1978).

The rumen is the principal fermentation site of a large number of predominantly anaerobic bacteria and protozoa. This is where cellulose is hydrolyzed into shorter polysaccharides and then converted to short-chain fatty acids such as acetic, propionic and butyric acids, which are used by the host animal. Some

bacterial species can utilize fatty acids and non-protein nitrogen such as ammonia to synthesize their amino acids and proteins. Thus, rumen microbes serve as significant sources of protein and other nutrients for the host animal. The function of the abomasum is largely similar to that of the stomach of non-ruminant mammals. The compartments before the abomasum facilitate (a) return of fibrous material to the mouth for further salivation and chewing and (b) utilization of cellulose and non-protein nitrogen.

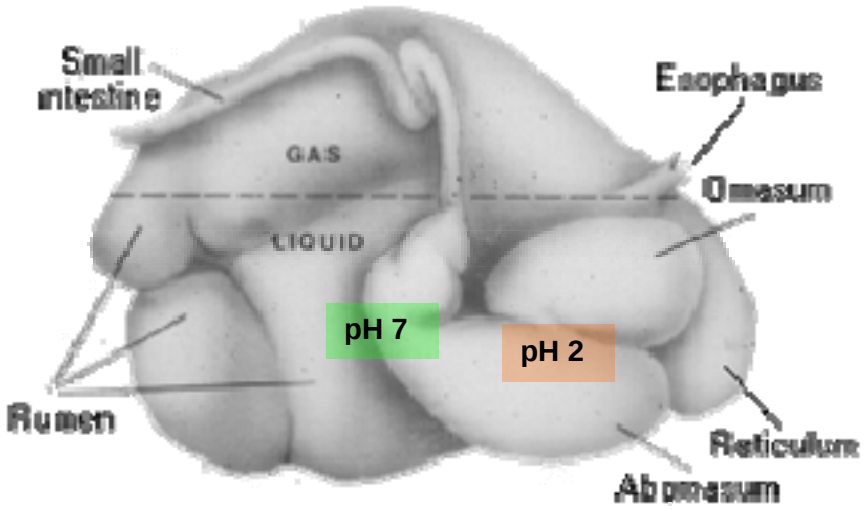

Figure 2.2 Ruminant stomach (from Meier, personal communication)

Vandamme and Ellis (2004) described the different parts of the gastro-intestinal tract of the cattle (Figure 2.3). In a ruminant digestive process, ingested feedstuffs are retained in the rumen for approximately 18 h and in the abomasum for approximately 30 min before moving into the upper intestinal tract. The rumen fluid has a pH within the range of about 5.5–7.0, and the abomasal fluid has a pH of about 2.0–3.0. This pH difference is the basis for developing a pH-dependent rumen-stable delivery system. As well as understanding the ruminant digestive process, it is necessary to understand the dynamics of ruminal motility and the effects of rumination on particle degradation. Pell *et al.* (1988) studied the movement of plastic pellets in the reticulo-rumen of cattle and found that particle dimension and specific gravity were important factors when designing a rumen-stable delivery system.

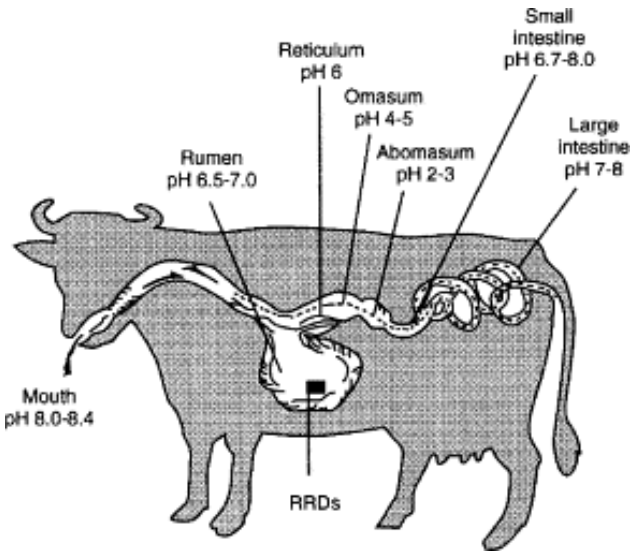

Figure 2.3 Schematic of the gastro-intestinal tract of the cattle (Vandamme and Ellis, 2004)

2.2.2 Post-ruminal delivery systems

A rumen-stable delivery drug system needs to meet the following major criteria (Wu, 1997):

1. Efficacy: the delivery system should provide rumen protection and post-ruminal bioavailability for the active ingredients. Ideally, nutrients, drugs and other active compounds should be fully protected from fermentation in the rumen and then be completely released and available for absorption or for performing their intended post-ruminal function.
2. Safety: the components used in formulating a rumen-stable delivery system must be safe for animals and humans. The chemical or other formulation processes must not generate undesirable residues in the finished product to cause safety concerns.
3. Cost: a rumen-stable delivery system must be cost-effective compared with low cost conventional systems. For example, feeding fish-meal increases the post-ruminal supply of methionine, lysine and other amino acids. If feed is supplemented with rumen-protected methionine and/or lysine, the same benefit must be achieved at a similar or lower cost.

The polymer used to formulate the rumen-stable delivery system must itself be suitable for formulation and must meet the following major criteria:

1. Inert, non-absorbable, unchanged in excretions in animals such as rats, dogs, cows and steers;
2. Non-mutagenic and non-teratogenic;
3. Produces no adverse effect following chronic feeding;
4. Thermally stable or non-degradable at temperatures experienced during process and storage;
5. Insoluble in rumen fluid, but soluble in abomasal fluid;
6. Soluble in common volatile organic solvents for coating applications.

2.3 OVERVIEW OF BIOMATERIALS

Biomaterials are substances other than the food or drug contained in therapeutic or diagnostic systems that are in contact with tissue or biological fluids. They have been used in many medical and/or pharmaceutical applications such as orthopaedic, dental or breast implants, artificial organs, pacemakers, sutures, vascular grafts, heart valves, intraocular and contact lenses, renal dialysers and other medical devices or controlled drug delivery systems (Langer, 2000; Castner *et al.*, 2002). A review indicates that biomaterials applications are involved in about 8000 kinds of medical devices, 2500 separate diagnostic products, and 40,000 different pharmaceutical preparations (Peppas & Langer, 1994; Langer and Peppas, 2003). Annual sales of advanced drug delivery systems using biomaterials in the United States exceed $100 billion and are rising rapidly (Langer, 2000).

2.3.1 Stimuli-sensitive biomaterials

Environmentally-sensitive polymers exhibit sharp changes in behaviour in response to an external stimulus such as pH, temperature, solvents, salts, electrical field, and chemical or biochemical agents (Qiu and Park, 2001). These “smart polymers” can be used for drug delivery.

Developing a glucose-sensitive insulin releasing system for diabetes therapy has been a long-standing challenge for biomedical engineers so it is not surprising that it has become a popular model for smart polymers. The specific release of the

required amount of insulin in response to changes in glucose levels can be designed in the form of a 'chemical valve' (Galaev and Mattiasson, 1999). Glucose oxidase can be immobilized on a pH-responsive poly(acrylic acid) layer grafted onto a porous polycarbonate membrane. Under neutral conditions, polymer chains are densely charged and have an extended conformation, preventing insulin transport through the membrane by blocking the pores. When exposed to glucose, the pH drops and the polymer chains become protonated and adopt a more compact conformation. The degree to which the pores are blocked is reduced and insulin is transported through the membrane.

2.3.2 Hydrogels

Hydrogels, which are three-dimensional, hydrophilic, polymeric networks that can imbibe large amounts of water or biological fluids, have been used extensively to develop smart drug delivery systems. The networks are composed of homopolymers or copolymers, and are insoluble due to the presence of chemical or physical crosslinks such as entanglements or crystallites (Peppas, 2003).

Polymers such as dextran, gelatine, amylose, cellulose, chitosan, fibrin, collagen, alginate, poly(vinyl alcohol), poly(hydroxyethyl methacrylate), poly(hydroxyethyl acrylate), poly(acrylic acid) (Carbopol), polyasparthydrazide, and poly(ethylene glycol) have been used to prepare hydrogels. A stepwise methodology is used to prepare hydrogel-based drug delivery systems (Figure 2.4). These delivery systems can be used for various sites such as oral, rectal, ocular, epidermal and subcutaneous application (Gupta *et al*., 2002).

2.3.3 Hydrophobic biomaterials

A wide range of hydrophobic biomaterials has been studied in drug delivery applications. The ethylene vinyl acetate copolymer series have been used in many successful products such as the early transdermal and ocular therapy systems (Peppas and Brannon-Peppas, 2003). Other hydrophobic polymers include polyethylene, polypropylene, various types of hydrophobic cellulose derivatives such as ethyl cellulose, etc.

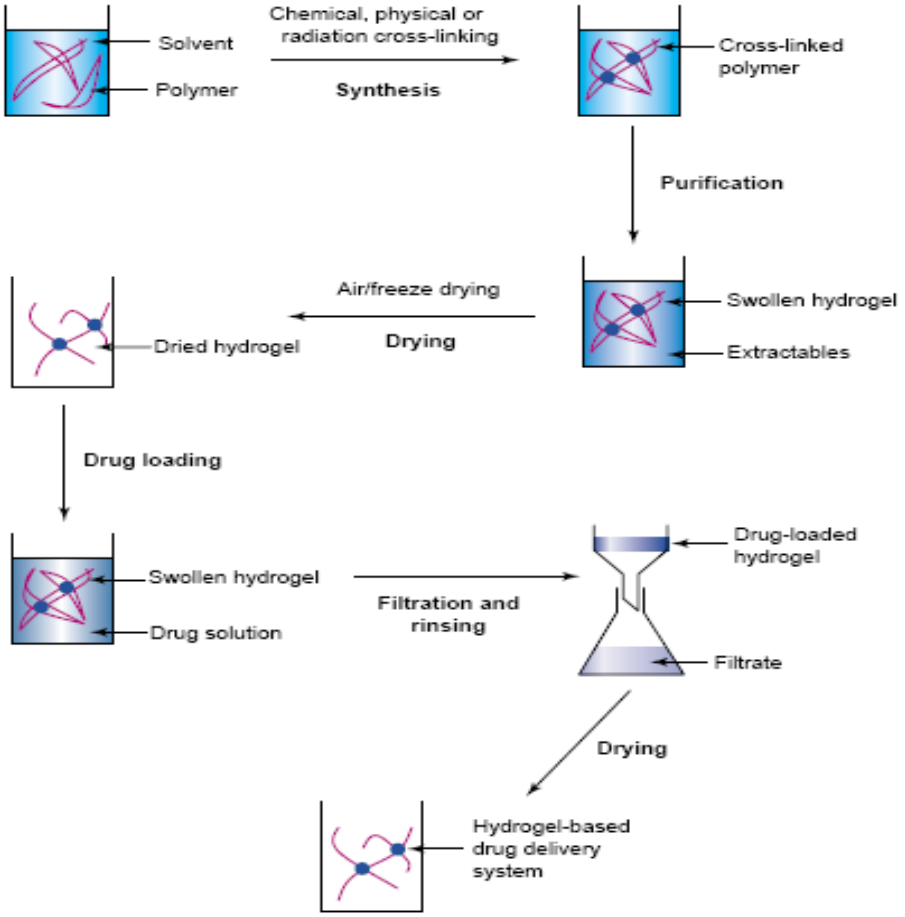

Figure 2.4 Method for preparing hydrogel-based drug delivery systems (Gupta *et al.*, 2002)

2.3.4 Hydrophilic biomaterials

Hydrophilic biomaterials are predominantly polymeric networks that swell in water or biological fluids without dissolving. Many natural polymers and their derivatives can be used including anionic polymers (hyaluronic acid, alginic acid, pectin, carrageenan, chondroitin sulphate, dextran sulphate), cationic polymers (chitosan, polylysine), amphipathic polymers (collagen and gelatine, carboxymethyl chitin, fibrin), and neutral polymers (dextran, agarose, pullulan) (Hoffman, 2002).

Many synthetic polymers have also been developed including (Hoffman, 2002): polyesters such as PEG-PLA-PEG, PEG-PLGA-PEG, PEG-PCL-PEG, PLA-PEG-PLA, PHB, poly(PEG/PBO terephthalate), and poly(PF-co-EG)-acrylate end groups; and other polymers such as PEG-bis-(PLA-acrylate), PEG-g-P(AAm-co-

Vamine), PAAm, PEG-CDs, poly(NIPAAm-co-AAc), poly(NIPAAm-co-EMA), PVAc/PVA, PNVP, poly(MMA-co-HEMA), poly(GEMA-sulphate), poly(AN-co-allyl sulfonate), and poly(biscarboxy-phenoxy-phosphazene).

Natural and synthetic polymers can also be combined. For example, poly(PEG-co-peptides), alginate-g-(PEO-PPO-PEO), alginate-acrylate, P(PLGA-co-serine), poly(HPMA-g-peptide), collagen-acrylate, poly(HEMA/Matrigel), and HA-g-NIPAAm (Hoffman, 2002).

2.3.5 Biodegradable polymers

Biodegradable polymers, by definition, change their chemical and potentially physical form when they contact the biological environment. Polymers which degrade by hydrolysis include poly(lactic acid), poly(glycolic acid), poly-(caprolactone), polyanhydride, poly(ortho ester), and polycyanoacrylate. Many poly(amino acids) such as poly(L-lysine), poly(L-arginine), poly(L-aspartic acid), poly(L-glutamic acid), and poly[N-(2-hydroxyethyl)-L-glutamine] can be degraded enzymatically (Peppas and Brannon-Peppas, 2003).

A wide range of synthetic polymers is available for designing biocompatible drug carriers. Polymers, such as poly(ethylene glycol), can be attached to drugs to either lengthen their lifetime or alter their immunogenicity. The polymers physically prevent cells and enzymes from attacking the drug. Poly(ethylene glycol) (PEG) is a biocompatible, non-toxic, non-immunogenic and water-soluble polymer much used in biomaterials, biotechnology and medicine. For example, PEG star polymer gels prepared by gamma-irradiation have been used for protein delivery with and without molecular imprinting (Peppas *et al*., 1999). Two types of novel degradable PEG hydrogels with potential utility as delivery carriers for bioactive drugs have also been synthesized and characterized (Zhao, 1998).

Murthy (2003) described a new class of versatile pH-responsive, endosomal disruptive polymeric carriers for biomolecules called ‘encrypted polymers’ (Figure 2.5). These polymers have a membrane-disruptive backbone with PEG grafts conjugated through acid-degradable linkers. Drug molecules and targeting

agents may be conjugated by acid-degradable linkages directly to the backbone or at the free ends of the PEGs. At pH 7.4, the polymers are PEGylated ('masked'). After endocytosis, the acid-degradable linker hydrolyses in the low pH environment of the endosome and the polymer backbone de-PEGylates ('unmasks'), which destabilises the endosomal membrane.

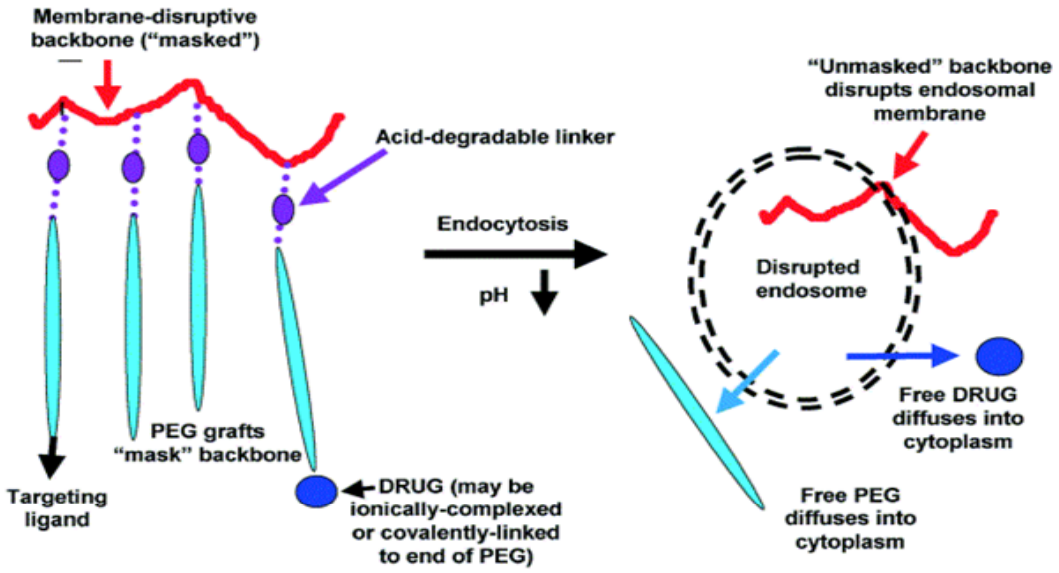

Figure 2.5 Action of an 'encrypted' polymeric drug carrier (Murthy, 2003)

2.4 POLYMERIC BIOMATERIALS AND THEIR APPLICATIONS

The purpose of this section is to review the properties, structure, and applications of polymeric biomaterials in detail. An overview of polymers used for drug delivery systems are described first and then several commonly used polymers are discussed.

The important components of living cell (protein, carbohydrates and nucleic acids) are all polymers. Polymeric materials have gained increasing interest during the 20th century and have been used in a vast number and wide range of applications. Table 2.2 summarises some commonly-used polymeric materials (Michigan State University, 2004).

Table 2.2 Some commonly-used polymers

Name(s)	Formula	Monomer	Properties	Uses
Polyethylene low density (LDPE)	$-(CH_2\text{-}CH_2)_n-$	ethylene $CH_2=CH_2$	soft, waxy solid	film wrap, plastic bags
Polyethylene high density (HDPE)	$-(CH_2\text{-}CH_2)_n-$	ethylene $CH_2=CH_2$	rigid, translucent solid	electrical insulation bottles, toys
Polypropylene (PP) different grades	$-[CH_2\text{-}CH(CH_3)]_n-$	propylene $CH_2=CHCH_3$	atactic: soft, elastic solid isotactic: hard, strong solid	similar to LDPE carpet, upholstery
Poly(vinyl chloride) (PVC)	$-(CH_2\text{-}CHCl)_n-$	vinyl chloride $CH_2=CHCl$	strong rigid solid	pipes, siding, flooring
Poly(vinylidene chloride) (Saran A)	$-(CH_2\text{-}CCl_2)_n-$	vinylidene chloride $CH_2=CCl_2$	dense, high-melting solid	seat covers, films
Polystyrene (PS)	$-[CH_2\text{-}CH(C_6H_5)]_n-$	styrene $CH_2=CHC_6H_5$	hard, rigid, clear solid soluble in organic solvents	toys, cabinets packaging (foamed)
Polyacrylonitrile (PAN, Orlon, Acrilan)	$-(CH_2\text{-}CHCN)_n-$	acrylonitrile $CH_2=CHCN$	high-melting solid soluble in organic solvents	rugs, blankets clothing
Polytetrafluoroethylene (PTFE, Teflon)	$-(CF_2\text{-}CF_2)_n-$	tetrafluoroethylene $CF_2=CF_2$	resistant, smooth solid	non-stick surfaces electrical insulation
Poly(methyl methacrylate) (PMMA, Lucite, Plexiglas)	$-[CH_2\text{-}C(CH_3)CO_2CH_3]_n-$	methyl methacrylate $CH_2=C(CH_3)CO_2CH_3$	hard, transparent solid	lighting covers, signs skylights
Poly(vinyl acetate) (PVAc)	$-(CH_2\text{-}CHOCOCH_3)_n-$	vinyl acetate $CH_2=CHOCOCH_3$	soft, sticky solid	latex paints, adhesives
cis-Polyisoprene natural rubber	$-[CH_2\text{-}CH=C(CH_3)\text{-}CH_2]_n-$	isoprene $CH_2=CH\text{-}C(CH_3)=CH_2$	soft, sticky solid	requires vulcanization for practical use
Polychloroprene (cis + trans) (Neoprene)	$-[CH_2\text{-}CH=CCl\text{-}CH_2]_n-$	chloroprene $CH_2=CH\text{-}CCl=CH_2$	tough, rubbery solid	synthetic rubber oil resistant

2.4.1 Polymers used for drug delivery systems

Using polymers for controlled release date back to the 1960s with silicone rubber (Folkman and Long, 1964) and polyethylene (Desai *et al*., 1965). Because these systems are not degradable, they need to be removed surgically, which limits their applicability. Using polymer microcapsules as delivery systems was reported as early as the 1960s (Chang, 1964). In the 1970s, biodegradable polymers were suggested as appropriate drug delivery materials because they did not need surgical removal and Mason *et al*. (1976) incorporated degradation by using a degradable polymer coating.

Polymeric materials generally release drugs by the following mechanisms: (1) diffusion of the drug species from or through the system; (2) a chemical or enzymatic reaction leading to degradation of the system, or cleavage of the drug from the system; and (3) solvent activation, either through osmosis or swelling of the system (Langer, 1990; Brannon-Peppas, 1997). Drug release is controlled by selecting properties such as polymer molecular weight, and microsphere size, distribution, morphology and make-up.

Because of the inherent diversity of structures, classifying polymers used in controlled release applications can be difficult. In broad terms, polymers may be classified as either biodegradable or non-biodegradable. Based on their behaviour in living tissue, polymeric biomaterials can be divided into two groups: biostable and biodegradable polymers.

Biostable polymers, typically polyethylene and poly(methyl methacrylate), should be physiologically inert in tissue conditions and able to maintain their mechanical properties for decades (Vainionpää, 1989). Biodegradable polymers are intended for temporary aids such as sutures, tissue-supporting scaffolds, and drug delivery devices (Mathiowitz *et al*., 1999). These polymers retain their properties for a limited period and then gradually degrade into soluble molecules that can be excreted from the body (Vainionpää, 1989).

2.4.2 Poly(amides)

Poly(amino acids) are the interesting class of poly(amides) for controlled release. The ability to manipulate synthetic amino acid sequences has maturated and used over the last two decades with new techniques and strategies continually being introduced. Nathan and Kohn (1994) gave an excellent review of the history of amino acid-derived polymers. Poly(amino acids) have been used predominantly to deliver low molecular weight drugs; they are usually tolerated when implanted in animals and are metabolized to relatively non-toxic products, which indicates good biocompatibility. However, their mildly antigenic nature makes widespread use uncertain. Another concern is the intrinsic hydrolytic stability of the amide bond, which requires enzymes for bond cleavage. This dependence generally results in poor controlled release *in vivo*.

Poly(amino acids) are highly insoluble, non-processible, and antigenic when the polymers contain three or more amino acids (Anderson *et al*., 1985). To solve these problems, "pseudo"-poly(amino acids) synthesized from tyrosine dipeptide were investigated (Kohn and Langer, 1987). These degradable polymers are derived from the polymerization of desaminotyrosyl tyrosine alkyl esters. Tyrosine-derived poly(carbonates) are readily processible polymers that support the growth and attachment of cells and have also shown a high degree of tissue compatibility. Tyrosine-derived poly(carbonates) are characterized by their relatively high strength and stiffness exceeding poly(esters) (Daniels *et al*., 1990; Engelberg and Kohn, 1991).

2.4.3 Poly(esters)

The family of poly(esters) has been by far the dominating choice for materials in degradable drug delivery systems. Copolymers derived from glycolic acid or glycolide, lactic acid or lactide, caprolactone, and 3- hydroxybutyrate have been given special attention. Poly(esters) are the best characterized and most widely studied biodegradable system. The synthesis of poly(esters) has received as much attention as the degradation of these materials. The predominant synthetic pathway for production of poly(esters) is from ring-opening polymerisation of the corresponding cyclic lactone monomer (Mathiowitz *et al.*, 1999). A patent for

using poly(lactic acid) (PLA) as a resorbable suture material was first filed in 1967 (Schmitt and Polistina, 1967). The mechanism of degradation in poly(ester) materials is classified as bulk degradation with random hydrolytic scission of the polymer backbone. Poly(esters) have been extensively employed in drug delivery applications and comprehensively reviewed (Lanza *et al*., 1997).

Studies in hydrolytic degradation for poly(esters) have focused on understanding the effects of changes in polymer chain composition. A distinguishable effect based on end group composition for poly(ester) degradation demonstrated that terminal carboxyl groups have a catalytic effect on hydrolysis for poly(glycolic acid) (Huffman, 1985). The commercial developmental process for formulating poly(esters) with selected drug candidates has been reviewed (Tracy, 1998).

2.4.4 Poly(ortho esters)

The motivation for designing poly(ortho esters) for drug delivery was the need to develop biodegradable polymers that inhibited drug release by diffusion mechanisms and allowed drug release only after the hydrolysis of polymer chains at the surface of the device.

Most research on poly(ortho esters) has focused on the synthesis of polymers by the addition of polyols to diketene acetals. For example, Heller *et al*. (1980) have described the synthesis and application of the 3,9-diethylidene-2,4,8,10-tetraoxaspiro[5.5]undecane (DETOSU)-based poly(ortho esters). A new class of poly(ortho esters) has been prepared by the reaction between a triol and an alkyl orthoacetate (Heller *et al*., 1990). A poly(ortho esters) prepared from 1,2,6-hexanetriol and trimethyl orthoacetate was investigated for the delivery of 5-fluorouracil and mitomycin C in glaucoma filtering surgery (Merkli *et al*., 1995; Zignani *et al*., 1997).

2.4.5 Poly(anhydrides)

Poly(anhydrides) undergo hydrolytic bond cleavage to form water-soluble degradation products that can dissolve in an aqueous environment, thus resulting

in polymer erosion. Poly(anhydrides) are believed to undergo predominantly surface erosion due to the high water lability of the anhydride bonds on the surface and the hydrophobicity that prevents water penetrating into the bulk (Leong, 1985).

Poly(anhydrides) are best formed into drug-loaded devices by compression-molding or microencapsulation because of their high melting temperatures. A wide variety of drugs and proteins, such as insulin, enzymes, and growth factors, have been incorporated into poly(anhydride) matrices and their in vitro and in vivo release characteristics evaluated. Leong *et al*. (1986) demonstrated that reaction of the poly(anhydrides) with drug molecules containing nucleophilic groups did not occur during fabrication using solvent-casting techniques or when low temperatures are maintained during compression moulding. Poly(anhydrides) used in oral delivery of insulin and plasmid DNA have been investigated (Mathiowitz *et al.*, 1997).

2.4.6 Polysulfone

Polysulfone (Figure 2.6) has excellent properties, such as good thermal, chemical, and mechanical stability (Allcock and Lampe, 1990). It is one of the most important polymeric materials used in the biomedical field and can be processed as an asymmetric membrane or hollow fibre with a dense skin layer (which determines the permeability for solutes) and a porous structure as a support. Applications include membranes for dialysis and hemodialysis. Polysulfone has been used to make ultrafiltration membranes and applications, processes and modifications have been reported (Ulbricht, 1996a, 1996b, 1998; Shim *et al.*, 1999).

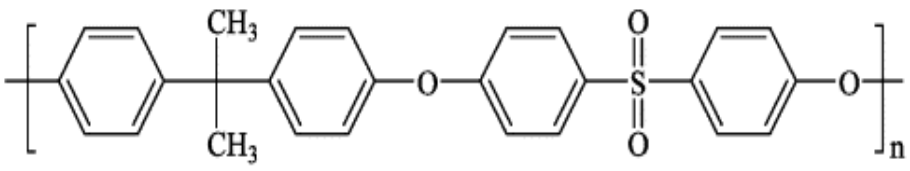

Figure 2.6 Chemical structure of polysulfone

To improve the biocompatibility of polysulfone, Ishihara *et al.* (1999) modified the polysulfone with 2-methacryloyloxyethyl phosphorylcholine (MPC). The amount of protein adsorbed on the PSF/MPC polymer blend membrane was significantly decreased with the increase in the MPC content. Higuchi *et al*. (2002) covalently conjugated polyvinyl pyrrolidone (PVP) to the surface of PSF membranes. These researchers suggested that the hydrophilic surface of the PVP-PSF membranes without ionic groups suppresses platelet adhesion to PVPPSF membranes and, furthermore, that the long hydrophilic side chain of PVP on PVP-PSF membranes contributes to the hydrophilic and hemocompatible wipers on the surface of the hydrophobic PSF membranes. Several researchers (Lu *et al.*, 2005; Park, 2006; Kung *et al.*, 2006) have reported on surface modification of polysulfone membranes by grafting copolymers such as poly(ethylene glycol), poly(acrylic acid), and poly(tert-butyl acrylate) to the membranes.

However, most applications of polysulfone focus on membranes. Only Gong *et al.* (2006) reported that polysulfone microcapsules containing triotylamine were prepared by a phase inversion method for separating organic acids.

2.4.7 Polystyrene

The chemical makeup of polystyrene is a long chain hydrocarbon with every other carbon connected to a phenyl group (Figure 2.7):

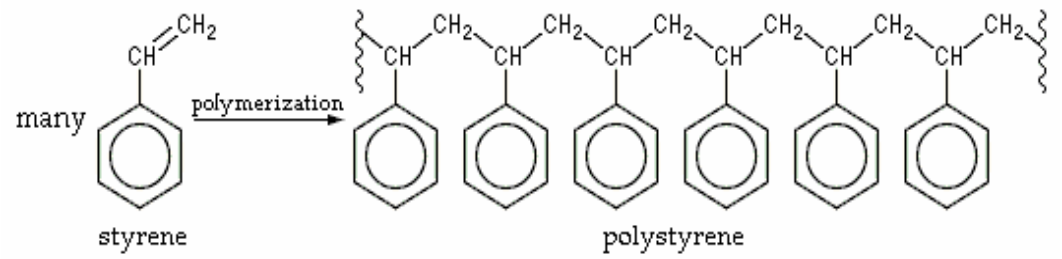

Figure 2.7 Chemical structure of polystyrene

Pure solid polystyrene is a colorless, hard plastic with limited flexibility. It can be cast into molds with fine detail. Polystyrene can be transparent or can be made to take on various colors. It is economical and is used for producing plastic model assembly kits, plastic cutlery, CD jewel cases, and many other objects where a

fairly rigid, economical plastic of any of various colors is desired. Petri dishes and other containers such as test tubes, made of polystyrene, play an important role in biomedical research and science. Polystyrene appears to be one of the best candidate polymers to be used in this study because it is low cost, easy to process, and has good mechanical property (Table 2.3).

Table 2.3 Properties of polystyrene

Density	1050 kg/m³
Electrical conductivity (σ)	10^{-16} S/m
Thermal conductivity	0.08 W/(m·K)
Young's modulus (*E*)	3000-3600 MPa
Tensile strength (σ_t)	46–60 MPa
Glass temperature	95°C
Melting point	240°C
Linear expansion coefficient (α)	8 x 10^{-5} /K
Water absorption (ASTM)	0.03–0.1 %

There are reports on preparing polystyrene copolymer microspheres using a solvent evaporation method. Ma *et al*. (1999) prepared polystyrene-poly(methyl methacrylate) (PSt-PMMA) composite microspheres by using the Shirasu porous glass membrane emulsification technique. Poly(styrene-co-butyl acrylate) microcapsule containing Fe_3O_4 (Omi *et al*., 2001) or TiO_2 (Supsakulchai *et al*., 2002) were reported. However, these products have brittle walls and the inside structure is honeycomb-like.

To date there are only a few reports on polystyrene microcapsules. Nishihama *et al*. (2004) prepared a column packed with microcapsules consisting of styrene-divinylbenzene copolymer and containing tri-*n*-octylmethylammonium chloride (TOMAC) or bis(2-ethylhexyl)phosphoric acid (D2EHPA) as the extractant. Yang *et al.* (2005) reported preparation of polystyrene microcapsules containing Aliquat 336 as a novel packing material in chromatography for separation of metal ions.

2.5 SURFACE MODIFICATION OF POLYMER MATERIALS

Surface modification to control surface properties is a useful way to obtain functional polymers. The surface modification technique is required to restrict the shallow depth of the modified layer from the surface. Generally, chemical reactions involving implantation of special elements (oxygen, nitrogen, fluorine atoms, etc.), moieties (hydroxyl, carbonyl, carboxylate, etc.), and grafting of special monomers are used in the modification process.

Many strategies can be used to modify polymer surfaces and can be grouped into two broad categories (Table 2.4) (Ratner, 1995):

1. Chemically or physically altering atoms or molecules in the existing surface (treatment, etching, chemical modification)
2. Coating the existing surface with a material having a new composition (solvent coating, thin film deposition by chemical vapour deposition, radiation grafting, chemical grafting, RF-plasmas).

2.5.1 Polymer brushes

Polymer brushes (or tethered polymers) describe an assembly of polymer chains tethered by one end to a surface or an interface (Milner, 1991). The tethering is sufficiently dense so the polymer chains are crowded and forced to stretch away from the surface or interface to avoid overlapping. Sometimes these chains are stretched much farther than the typical unstretched size of a chain. These stretched configurations are found under equilibrium conditions so neither a confining geometry nor an external field is required. The stretching of the polymer chains to the grafting surface is quite different from the typical behaviour of flexible polymer chains in solution, where chains adopt a random-walk configuration. The deformation of densely tethered chains affects many aspects of their behaviour and results in many novel properties of polymer brushes (Szleofer and Carignano, 1996).

Table 2.4 Physical and chemical surface modification methods

Methods	Examples
Non-covalent coatings	Solvent coating
	Langmuir-Blodgett film deposition
	Self-assembled layers (e.g. thiol/Au)
	Surface active additives
	Vapour deposition of carbons and metals
	Vapour deposition of parylene (p-xylylene)
Covalently attached coatings	Radiation grafting (Electron accelerator and Gamma)
	Electron beam-induced grafting
	Photografting (UV and visible sources)
	Plasma (gas discharge) (RF, microwave, acoustic)
	Gas phase deposition
	- ion beam sputtering - chemical vapour deposition
	Chemical grafting (e.g. ozonation + grafting)
	Silanisation
	Biological modification (biomolecule immobilisation)
Modifying the original surface	Ion beam etching (e.g. argon, xenon)
	Ion beam implantation (e.g. nitrogen)
	Plasma etching (nitrogen, argon, oxygen, water vapour)
	Corona discharge (in air)
	Electron beam treatment
	Ion exchange
	UV irradiation
	Chemical reaction
	- non-specific oxidation (e.g. ozone)
	- functional group modifications (oxidation, reduction)
	- addition reactions (e.g. acetylation, chlorination)
	Conversion coatings (phosphating, anodisation)

Polymer brushes are a central model for many practical polymer systems such as polymer micelles, block copolymers at fluid–fluid interfaces (e.g. microemulsions and vesicles), grafted polymers on a solid surface, adsorbed diblock copolymers, and graft copolymers at fluid–fluid interfaces (Figure 2.8). All these systems have a common feature: the polymer chains exhibit deformed configurations. When

the polymer brushes are in a suitable solvent, they try to avoid contact with each other and maximise contact with the solvent molecules. With solvent absent (melt conditions) the polymer chains must stretch away from the interface to avoid overfilling the incompressible space (Zhao, 1999).

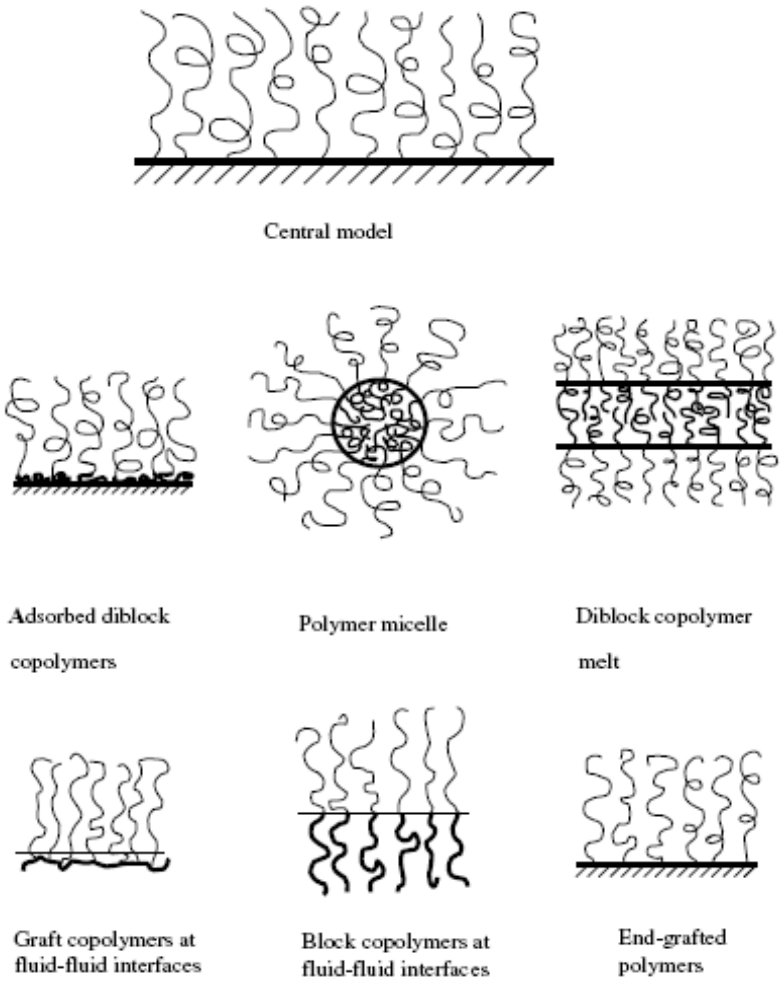

Figure 2.8 Polymer systems with polymer brushes (Zhao, 1999)

The polymer chains in the polymer brushes may be tethered to a solid substrate surface or to an interface between two liquids, between a liquid and air, between melts, or between solutions of homopolymers. The tethering on the surface or interface can be reversible or irreversible. The polymer chains in polymer brushes can be chemically bonded to a solid substrate or may be just adsorbed onto the surface. Physisorption on a solid surface is usually achieved by block copolymers where one block interacts strongly and another block interacting weakly with the substrate. For interfaces between fluids, the attachment may be achieved by

similar adsorption mechanisms in which one part of the chain prefers one medium and the rest of the chain prefers the other.

Polymer brushes attracted attention in the 1950s, when it was found that grafting polymer molecules to colloidal particles very effectively prevented flocculation (Van der Waarden, 1950; Mackor, 1951). The attached polymer chains preferred the suspension solvent to the colloidal particle surface. Because brushes of two approaching particles resisted overlapping colloidal stabilization is achieved. Repulsive force between brushes arises from the high osmotic pressure inside the brushes. Polymer brushes were subsequently found to be useful in other applications such as polymer surfactants (Milner, 1991), polymer compatibilisers (Milner, 1991), lubricants (Joanny, 1992), new adhesive materials (Ji, 1993), protein-resistant biosurfaces (Amiji and Park, 1993), and chromatographic devices (Van Zanten, 1994). Polymer brushes that have low critical solution temperature (LCST) properties exhibit different wetting properties above and below LCST temperature (Takei *et al*., 1994).

A very promising field, which has been extensively investigated, is using polymer brushes as chemical gates. Ito *et al.* (1997) described pH-sensitive, photo-sensitive, oxidoreduction sensitive polymer brushes covalently tethered on porous membranes, which are used to regulate the liquid flow rate through porous membranes. Suter *et al*. (1999) prepared polystyrene brushes on high surface area mica (group of phyllosilicate mineral) to fabricate organic-inorganic hybrids. Cation-bearing peroxide free-radical initiators were attached to mica surfaces via ion exchange and used to polymerize styrene. This process is important in the field of nanocomposites.

In terms of polymer chemical compositions, polymer brushes tethered on a solid substrate surface can be classified into several groups: (1) homopolymer brushes (single repeating unit); (2) mixed homopolymer brushes composed of two or more types of homopolymer chains (Soga *et al*., 1996); (3) random copolymer brushes consisting of two different repeat units which are randomly distributed along the polymer chain (Mansky *et al*., 1997); and (4) block copolymer brushes where the assembly of tethered polymer chains consist of two or more homopolymer chains

covalently connected to each other at one end (Zhao and Brittain, 1999). Homopolymer brushes can be further divided into neutral and charged polymer brushes. Brushes may also be classified in terms of polymer chain rigidity as flexible, semiflexible, and liquid crystalline polymer brushes.

Generally, there are two main ways to fabricate polymer brushes: physisorption and covalent attachment (Figure 2.9). For polymer physisorption, block copolymers adsorb onto a suitable substrate, with one block interacting strongly with the surface and the other block interacting weakly with the substrate. Covalent attachment can be accomplished by either "grafting to" or "grafting from" approaches. In a "grafting to" approach, preformed end-functionalized polymer molecules react with an appropriate substrate to form polymer brushes. The "grafting from" approach is a more promising method for synthesising polymer brushes with a high grafting density. It can be accomplished by treating a substrate with plasma or glow-discharge to generate immobilized initiators followed by polymerisation.

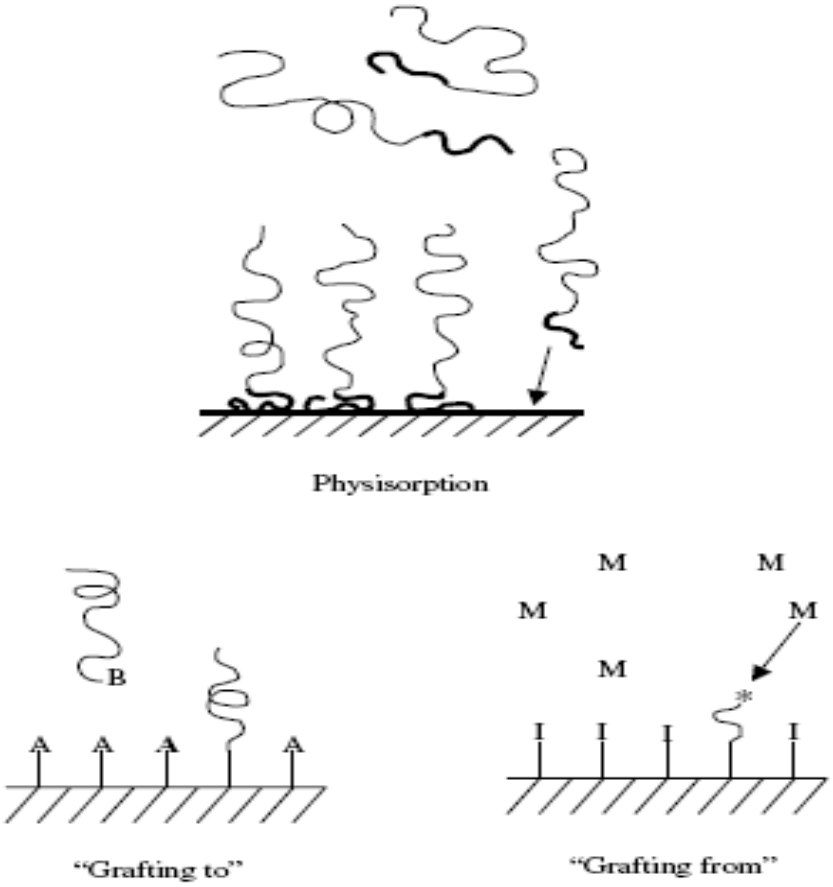

Figure 2.9 Preparing polymer brushes by physisorption or grafting

2.5.2 Plasma-induced grafting

Plasma polymerisation technique has received increasing interest. Plasma conditions obtained through slow discharge offer the same possibilities as ionizing radiation (Yamaguchi *et al*., 1994; Wenzel *et al*., 2000). The main processes in plasmas are electron-induced excitation, ionization and dissociation. The accelerated electrons from the plasma have sufficient energy to cleave chemical bonds in the polymeric structure, to form macromolecule radicals, which subsequently initiate graft co-polymerisation.

Four different techniques have been used to generate plasmas suitable for organic reactions (Hollahan, 1974):

1. Silent discharges
2. Direct-current and low-frequency glow discharges
3. Radio-frequency discharges (high-frequency)
4. Microwave discharges

Gancarz *et al*. (1999) used three different methods to modify polysulfone membranes with acrylic acid (AAc) using plasma-initiated graft polymerisation (both in solution and vapour phase) and plasma polymerisation: (1) grafting in solution; the plasma-treated polymer membrane was exposed to air for 5 min and dipped into deaerated aqueous solution of monomer; (2) grafting in vapour phase; when Ar plasma treatment on polymers was completed, the argon flow was stopped and monomer vapour introduced into the chamber; and (3) plasma polymerisation, where a polymer membrane was placed in a plasma reactor, the reactor was evacuated and Ar was introduced and vapours of monomer were introduced to give a desired pressure. Plasma polymerisation of AAc gave a layer of material very similar to pure poly(acrylic acid) (PAA). This layer was, however, loosely packed and could be easily removed from the membrane, leaving only a small amount of AAc permanently grafted onto the surface. Of the above three methods, polysulfone membrane with AAc plasma-initiated grafting in vapour phase of monomer appeared to have the most promising filtration properties.

Poncin-Epaillard *et al*. (1997) investigated morphologic transformation of isotactic PP by a cold nitrogen plasma treatment. Reactive species of the nitrogen were induced by plasma formation of macroradicals onto the polypropylene surface. Besides surface modifications, plasma treatment under certain conditions can increase film crystallinity, and even induce new crystallization with a concentration gradient depending on film thickness. There was a relationship between the crystallinity change and the plasma emission characteristics, including vacuum ultraviolet (VUV) radiation. Hydrogen plasma having the more important VUV emission affects more strongly the crystallinity of polypropylene film. During plasma treatment, two types of crystallization occurred, smectic phase to α phase, and amorphous to smectic phase. These respective proportions depended on the film thickness.

Oehr *et al*. (1999) investigated the effects of gas type to graft AAc and glycidyl methacrylate monomer onto a polymer surface. Grafting was carried out in the gas phase immediately after the plasma reaction. Activation using hydrogen was found to be successful for halogenated polymers because hydrogen atoms, together with halogens, formed stable volatile molecules that were easily pumped away from the reaction zone to leave a surface with many radical sites for grafting. The resulting interface for PTFE consisted of 9% carboxyl carbon atoms. In the presence of oxygen, no grafting was observed for poly(vinylidene fluoride) (PVDF); instead, the surface was mainly etched and not activated.

Under a similar distribution of supplied power, antenna coupling microwave plasma, with bias plates as capacitor containing radio-frequency plasma, gives ten times more in plasma ionization density. Consequently, from the physical chemistry perspective, antenna coupling microwave plasma source is relatively efficient for modifying the polymer matrix. Chen-Yang *et al*. (2000) modified the surface of expanded PTFE sheets using CO_2 cold plasma generated by an antenna coupling guided microwave. The PTFE sheet was effectively activated at the surface, which promoted the penetration capability of AAc monomer as well as monomer graft polymerisation. The plasma-induced PTFE surfaces had little structural alteration, and morphology of the nodes and stretched fibrils in the polymer also remained unchanged.

N-acryloylglycine (NAG) and diethylenetriaminepentaacetic acid (DTPA) with specific chelating properties were grafted onto polypropylene surface to synthesis of a new chelating membrane by different routes using nitrogen plasma (Poncin-Epaillard, 2000). After plasma treatment, the PP film was immediately dipped into an aqueous solution of NAG under nitrogen to minimise oxidation. The grafting reaction was performed at elevated temperature with Mohr's salt as homopolymerisation inhibitor. For the coupling reaction of DTPA, the plasma-treated PP film was immediately dipped into a solution of DTPA anhydride in dimethyl sulfoxide, also under nitrogen to minimize oxidation. Both the grafted polypropylene films had increased selectivity for silver ions, and consequently for soft metallic ions. The capacity of DTPA grafted films was lower than that of polyNAG, regardless of the density of amino groups attached onto the surface.

2.5.3 Radiation grafting

Radiation grafting can also proceed through an ionic mode, with the ions formed through high-energy irradiation. In the radiation technique, the presence of an initiator is not essential but the medium is important in this case. For example, peroxides may form on the polymer if irradiation is carried out in air. The lifetime of the free radical depends upon the nature of the backbone polymer (Bhattacharya *et al*., 1998; Chen *et al*., 2003).

2.5.4 Chemical methods

There are two main methods for chemical grafting: free radical and ionic grafting. The role of the initiator is very important because it determines the path of the grafting process. As well as the general free-radical mechanism, grafting in the melt and atom transfer radical polymerisation (ATRP) are other interesting techniques to carry out grafting.

2.5.4.1 Free-radical grafting

Free radicals are produced from the initiators and transferred to the substrate to react with the monomer to form the graft co-polymers. The free radicals can be produced by indirect or direct methods.

Free-radical sites may be generated on a polymeric backbone by direct oxidation of the backbone by certain transition metal ions (e.g. Ce^{4+}, Cr^{6+}, V^{5+}, Co^{3+}). The redox potential of the metal ions is the important parameter in determining the grafting efficiency. In general, metal ions with low oxidation potential give better grafting efficiency. The proposed mechanism involves forming of a metal ion–polymer chelate complex intermediate. For example, ceric ion is known to form a complex with hydroxyl groups on a polymeric backbone, which can dissociate via one electron transfer to give free radicals (Hsueh, 2003; Zhang, 2003). Grafting by ATRP of polymers including poly(vinyl chloride) (PVC), polyisobutene, polyethylene, and ethylene-*co*-vinyl acetate co-polymer has also reported (Paik *et al*., 1998; Hong *et al*., 2001; Matyjaszewski *et al*., 2000; Garcia *et al*., 2002).

2.5.4.2 Living polymerisation

Living polymerisation methods have developed to provide a potential for grafting reactions. Szwarc *et al*. (1998) proposed that the most plausible definition of a 'living polymer' is a chemical grafting that retains its ability to propagate for a long time and grow to a desired maximum size while its degree of termination or chain transfer is still negligible. Controlled free-radical polymerisations combine features of conventional free-radical and ionic polymerisations. Conventional free-radical polymerisation requires continuous initiation, with termination of the growing chain radicals in coupling or disproportionation reactions. This produces a mix of unreactive polymers, time-invariant degrees of polymerisation, and broad molecular weight distributions. Living polymerisation, on the other hand, provides living polymers with regulated molecular weights and low polydispersities (Russell, 2002).

2.5.4.3 Ionic grafting

Grafting can also proceed through an ionic mode. Alkali metal suspensions in a Lewis base liquid, organometallic compounds and sodium naphthalenide are useful initiators for this method. Alkyl aluminium (R_3Al) and the backbone polymer in the halide form (ACl) interact to form carbonium ions along the

polymer chain, which leads to copolymerisation. The reaction proceeds through a cationic mechanism:

$$ACl + R_3Al \rightarrow A^+R_3AlCl^-$$

$$A^+ + M \rightarrow AM^+\text{-}M \rightarrow \text{graft co-polymer}$$

Cationic catalyst BF_3 can also be used.

Grafting can also proceed through an anionic mechanism. Sodium ammonia or alkali metal methoxide form the alkoxide of the polymer ($PO^- Na^+$), which reacts with monomer to form the graft co-polymer:

$$P\text{-}OH + NaOR \rightarrow PO^- Na^+ + ROH$$

$$PO^- + M \rightarrow POM^- \text{-}M \rightarrow \text{graft co-polymer}$$

2.5.5 Photochemical grafting

The McMaster Membrane Research Group (Jiang, 1999; Mika *et al*., 1999) prepared a series of pore-filled membranes containing poly(4-vinylpyridine) (P4VP) and poly(4-vinylpyridium salts) by grafting methods, which involved inserting monomers such as 4-vinylpyridine (4VP), and a photo initiator (benzoin ethyl ether) within the pores of a polypropylene or polyethylene membrane followed by UV-initiated polymerisation. The poly(4-vinylpyridine) grafted to the substrate membrane can be protonated or alkylated to produce weak or strong base anion-exchange membranes. Simons and Dickson (1993) made P4VP-filled membranes by photografting P4VP to a polypropylene substrate membrane but did not examine the effect of converting the grafted P4VP to its protonated or alkylated forms (the salt forms).

2.5.6 Crosslinking

Mika *et al*. (1995) produced durable P4VP salt-filled membranes by introducing a crosslinking agent to 4-vinylpyridine during *in situ* polymerisation to form pore-filled membranes. It is likely that grafting is still occurring in these reactions. The incorporated crosslinked P4VP can also be converted into its salt form.

Kapur *et al*. (1996) showed that grafting was not required and that the crosslinked polyacrylamide hydrogel formed within pores of a poly(vinylidene fluoride) membrane was anchored by physical entanglement of the gel polymer materials with the host. In fact, Cussler *et al*. (1992) had earlier reported that pore-filled membranes could be produced by crosslinking pre-formed linear polymers such as polyvinyl alcohol (PVA) in the pores of a substrate membrane.

The McMaster Membrane Group (Childs *et al*., 2001; Mika *et al*., 2002) has also shown that stable pore-filled membranes can be produced by crosslinking a pre-formed polymer. This crosslinking reaction entangles and "locks" the guest polymers within the pores of the substrate membrane provided the degree of crosslinking is above a threshold value. They tested different membranes under pressure to determine hydrodynamic permeabilities and salt separation properties of membranes. There was a good correlation between hydrodynamic permeability and gel polymer concentration irrespective of the gel polymer chemistry in the pore-filling gels. Membranes with different pore-filling gels, whether positively or negatively charged, followed the same relationship. The separation properties of the membranes are very good and the salt rejection was found to be practically constant over a wide range of permeabilities.

2.6 MICROENCAPSULATION TECHNIQUES

Microencapsulation is a process in which small particles or droplets are entrapped by a coating to give the particles with many useful properties (Mathiowitz, 1999). In a relatively simplistic form, a microcapsule is a small sphere with a uniform wall around it. The material inside the microcapsule is referred to as the core, internal phase, or fill, whereas the wall is sometimes called a shell, coating, or membrane. There are two main encapsulation techniques, chemical and physical. The chemical technique involves polymerisation whilst the microcapsules are being manufactured, whilst the physical technique involves controlled precipitation of a polymeric solution, which produces physical changes. The term microsphere is often used synonymously with microcapsule. However, these two terms have different meanings in controlled release technology, which are also discussed in Chapter Four. A microcapsule is a system that contains a well-

defined core and a well-defined shell. The core can be solid, liquid, or gas and the shell is made of a continuous, porous, or nonporous, polymeric phase. On the other hand, a microsphere is a structure made of a continuous phase of one or more miscible polymers in which particulate drug are dispersed. Different encapsulation result in either a microcapsule or a microsphere.

It is difficult to classify encapsulation methods because specific techniques can be hybrids of two or more methods or can use different mechanisms simultaneously. Also many names have changed throughout the years (e.g., solvent evaporation has also been called double emulsion). Table 2.5 summarises the different techniques. Some reviews on aspects of microspheres for drug delivery are available (Jalil and Nixon, 1990; Mathiowitz, 1999; Kawaguchi, 2000; Tamber *et al.*, 2005).

Table 2.5 Microencapsulation techniques

Process	Coating material	Suspending medium
Coacervation	Hydrophobic polymers	Organic
Complex coacervation	Water-soluble polyelectrolyte	Aqueous
Hot melt	Hydrophilic or hydrophobic polymers	Aqueous/organic
Interfacial polymerisation	Water-soluble and insoluble monomers	Aqueous/organic
Phase separation	Hydrophilic or hydrophobic polymers	Aqueous/organic
Salting-out	Water-soluble polymers	Aqueous
Solvent evaporation	Hydrophilic or hydrophobic polymers	Organic/aqueous
Solvent removal	Hydrophilic or hydrophobic polymers	Organic
Spray-drying	Hydrophilic or hydrophobic polymers	Air, nitrogen
Thermal denaturation	Proteins	Organic

2.6.1 Manufacturing techniques

2.6.1.1 Interfacial polymerisation

This process involves condensation polymerization of two monomers at the interface of the organic and aqueous phases. The interfacial reaction occurs rapidly and is considered a rapid means of preparing microcapsules. Mathiowitz and Cohen (1989) prepared and investigated polyamide microcapsules using this

technique. Alexandridou *et al*. (1995) reported synthesis of oil-containing microcapsules by interfacial polymerisation and their electrolytic codeposition. Chu *et al*. (2001, 2003) produced thermosensitive core-shell microcapsules made of a porous polyamide membrane and grafted poly(N-isopropylacrylamide) chains into the membrane pores, which acted as thermoresponsive gates.

Interfacial polymerization is one of the simplest *in situ* polymerisation techniques and it almost always produces a microcapsule. However, studies on its application are lacking.

2.6.1.2 Solvent evaporation

In this technique, polymer is dissolved in a suitable water-immiscible solvent and the therapeutic is dispersed or dissolved in this solution. The resultant mixture is then emulsified in an aqueous continuous phase to form discrete droplets. To form microspheres, the organic solvent must first diffuse into the aqueous phase and then evaporate at the water/air interface. As solvent evaporation occurs, the microspheres harden and free-flowing microspheres can be obtained after filtration and drying.

The solvent evaporation method has been used extensively to prepare PLA and PLGA microspheres containing many different drugs (Jalil and Nixon, 1989, 1990; Huang *et al*., 1997; Atkins *et al*., 1998; Edlund and Albertsson, 1999; Oh *et al*., 1999; Pistel *et al*., 1999; Bai *et al*., 2001). Variables that can influence microspheres properties including: drug solubility, internal morphology, solvent type, diffusion rate, temperature, polymer composition and viscosity, and drug loading. Microsphere size can be affected by polymer concentration in the second emulsion, temperature, viscosity, the stirring rate in the second emulsion step, and the amount of emulsifier used. Increasing the polymer concentration in the second emulsion usually increases sphere size (Yan *et al*., 1994; Ghaderi *et al*., 1996; McGee *et al*., 1997; Schlicher *et al*., 1997; Lin and Vasavada, 2000).

The effectiveness of the solvent evaporation method to produce microspheres depends on successful entrapment of the active agent within the particles.

Therefore, this process is most successful with drugs that are either insoluble or poorly soluble in the aqueous medium that makes the continuous phase. Many types of drugs with different physical and chemical properties have been formulated into polymeric systems, including anti-cancer drugs, narcotic agents, local anesthetics, steroids, and fertility control agents (Bodmeier and McGinity, 1988a, b; Li *et al*., 1995a, b; Ghaderi *et al*., 1996).

Yang *et al*. (2000) used scanning electron microscopy (SEM) to show that sphere size was temperature dependent; larger spheres were produced at the lower and higher temperatures whereas smaller spheres were produced at intermediate temperatures. Once again, different mechanisms dominated microsphere formation at different temperatures. At lower temperatures, the solution's higher viscosity resulted in formation of larger spheres; this has also been confirmed by other researchers (Jeyanthi *et al*., 1997). Larger spheres were obtained at higher temperatures due to the higher rate of solvent evaporation, which resulted in higher solvent flow pressure moving more material from the sphere centre outward (Yang *et al*., 2000).

2.6.1.3 Hot melt microencapsulation

Mathiowitz and Langer (1987) used this method to prepare microspheres of polyanhydride copolymer of poly[bis(p-carboxy phenoxy) propane anhydride with sebacic acid. The polymer is first melted and then mixed with solid particles of the drug that have been sieved to less than 50 μm. The mixture is suspended in a non-miscible solvent (such as silicone oil), continuously stirred, and heated to 5°C above the melting point of the polymer. Once the emulsion is stabilised, it is cooled until the polymer particles solidify. The resulting microspheres are washed with petroleum ether. The primary objective for developing this method is to have a microencapsulation process suitable for water-labile polymers, e.g. polyanhydrides. Microspheres with 1–1000 μm in diameter can be obtained and the size distribution can be easily controlled by altering the stirring rate.

2.6.1.4 Solvent removal

This non-aqueous method of microencapsulation is particularly suitable for water-labile polymers such as the polyanhydrides. The drug is dispersed or dissolved in a solution of the selected polymer in a volatile organic solvent such as methylene chloride. This mixture is then suspended in silicone oil containing Span 85 and methylene chloride. Petroleum ether is then added and stirred until the solvent has been extracted into the oil solution. The resulting microspheres can then be dried under vacuum (Carino *et al*., 1999).

Mathiowitz *et al*. (1990) used this method to develop an improved technique of encapsulating insulin. The polymer was dissolved in methylene chloride, the desired amount of drug was added and then the mixture was suspended in silicone oil containing Span 85 and methylene chloride. After pouring the polymer solution into the silicone oil, petroleum ether was added and the mixture was stirred until the methylene chloride was extracted into the oil solution and sufficient microcapsule hardening was achieved.

2.6.1.5 Hydrogel microspheres

Microspheres made of gel-type polymers such as alginate are produced by dissolving the polymer in an aqueous solution, suspending the active ingredient in the mixture and then extruding the mixture through a precision device to produce microdroplets, which fall into a slowly stirred hardening bath. The hardening bath usually contains calcium chloride solution and the divalent calcium ions crosslink the polymer to form gelled microspheres. The method is "all-aqueous", which avoids having residual solvent in the microspheres. Lim and Moss (1981) developed this method to encapsulate live cells, because the mild conditions will not kill the cells. The surface of these microspheres can be further modified by coating them with polycationic polymers such as polylysine. The particle size of microspheres can be controlled by using various size extruders or by varying the polymer solution flow rates.

2.6.1.6 Spray drying

In this process, the drug is dissolved or dispersed in the polymer solution and then spray dried. The quality of spray-dried microspheres can be improved by adding plasticizers such as citric acid, which promote polymer coalescence on the drug particles and hence promote formation of spherical and smooth-surface microspheres. The microsphere size can be controlled by spray drying conditions (nozzle size, spraying rate, drying temperature, and feed rate of the polymer drug solution). This microencapsulation method is less dependent on the solubility characteristics of the drug and polymer, and is simple, reproducible, and easy to scale up (Bodmeier and Chen, 1988). Mathiowitz *et al*. (1992) used this technique to examine its influence on crystallinity, external morphology, and release kinetics of polyanhydrides.

2.6.1.7 Phase inversion

The process involves adding the drug to a dilute polymer solution (usually 1–5%, w/v in methylene chloride). The mixture is poured into an unstirred bath of a strong non-solvent at a solvent to non-solvent ratio of 1:100, which result in spontaneous production of microspheres through phase inversion. The microspheres in the size range of 0.5–5.0 μm can then be filtered, washed with petroleum ether and dried with air (Chickering *et al.*, 1996). This is a simple, fast, and easy to scale up process of microencapsulation with relatively little loss of polymer and drug.

2.6.2 Preparative routes

2.6.2.1 Using linear polymers

Preparing microspheres from linear polymers can be advantageous because of the wide range of commercially-available polymers. Existing polymers are manufactured into microspheres by solidification of emulsion, coacervation, solvent evaporation, spray drying, etc. (Kawaguchi, 2000). This microsphere preparation route is also useful for naturally occurring polymers (such as chitin, chitosan, and cellulose) and for polymers that cannot be made by emulsion processes. For example, biocompatible polylactide (PLA) and polyglycolide

(PGA) are usually obtained from anionic polymerisation instead of free radical methods. Kawaguchi (2000) reviewed functional polymer microspheres and their properties, medical and biomedical applications.

Some commonly used microsphere preparation methods are the solvent evaporation (or the double emulsion) technique and the spray drying technique. These methods are also described in a review on bioadhesive microspheres (Vasir et al., 2003). Pavanetto *et al*. (1993) evaluated spray drying methods used for preparing polylactide and poly(lactide-co-glycolide) microspheres. Witschi and Doelker (1998) compared the properties of microparticles prepared by the solvent removal technique or the spray drying technique. They used five polymers to prepare peptide loading microparticles and compare their degradation rate during *in vitro* testing. Berkland *et al*. (2001) also described spay drying techniques for the formation of monodisperse spheres.

2.6.2.2 Polymerising monomers

Although most microspheres used for drug delivery are prepared from linear polymers, preparing microspheres from monomers is still important. This process involves polymerising colloidal monomers dispersed in a liquid with opposite phase solubility. Spherical droplets are formed by oil-soluble organic monomers dispersed in aqueous media (oil-in-water, O/W) or by water-soluble monomers dissolved in water dispersed in an organic medium (water-in-oil, W/O). Various methods, including emulsion, suspension, and dispersion techniques, can be used to polymerise the dispersed monomers (Piirma, 1985). Emulsions are typically used to form uniform nanometre-sized spheres (10–1000 nm). Typically, a hydrocarbon monomer is dispersed in water with a water-soluble initiator. The resulting polymer beads can be so uniform that they may diffract visible light (Weissman *et al*., 1996).

Dispersion polymerisation produces 0.5–10 μm diameter particles and all the reagents including monomer, initiator, and stabilizer (often an organic polymer consisting of hydrophobic and hydrophilic parts) are dissolved in an organic medium. Since the initiator is soluble inside the monomer, polymerisation occurs

inside the monomer droplets. The polymer beads (insoluble in the organic solvent) precipitate and the stabilizer prevents bead flocculation (Strover and Li, 1996). Since 1990s, there has been much work on dispersion polymerisation in supercritical CO_2, which may be beneficial to medical applications because no toxic solvents are involved (Benedetti *et al*., 1997; Canelas and DeSimone, 1997).

Suspension polymerisations are typically used for micron-sized particles (50–500 μm). The monomer is dispersed in a water phase with a stabilizer and the initiator is soluble in the monomer phase, where polymerisation occurs. The size and quantity of the particles is determined by the size and quantity of dispersed monomer droplets and the mechanical stirring speed (Piirma, 1985).

Ruckenstein and Hong (1995) obtained uniform polymer beads on the millimetre scale by sedimentation polymerization, which involves the polymerizing aqueous monomer droplets rising through hot paraffin oil. Amsden (1999) developed a method for forming millimetre-sized beads using a solvated linear polymer. Droplets of polymer in organic solution were added to poly(vinyl alcohol) (as stabilizer)/water solution; to produce uniform-sized beads. Some of polymerising monomers techniques are summarised in Table 2.6.

Table 2.6 Particle sizes obtained from various bead-forming techniques.

Preparative methods	Size range	Reference
Emulsion polymerisation	0.01–1 μm	Weissman *et al*., 1996
Dispersion polymerisation	0.5–10 μm	Strover and Li, 1996
Suspension polymerisation	50–500 μm	Piirma, 1985
Sedimentation polymerisation	mm sizes	Amsden, 1999

2.7 RESEARCH PLAN

2.7.1 Objectives

The objectives of this thesis are to investigate a post-ruminal controlled delivery system based on polymeric microencapsulation. Rumen fluid has a pH of 5.5–7.0 (Section 2.2.1) and the abomasum fluid has a pH of 2.0–3.0. This pH difference

is the basis for developing a pH-dependent rumen-stable delivery system. The research examines ways to develop reversible and switchable drug protection technologies using smart polymers that respond to changes of pH in the *in vivo* environment. This protection will stop active ingredient breaking down or being absorbed in undesired sites in the body whilst allowing the drug to be absorbed at other sites. This means the drug can either be protected while it passes through harsh microbial environments (rumen) or customised to maximise uptake rates at a specific absorption site. The microcapsules must have a thin shell strong enough to withstand harsh physical conditions and a large hollow centre, which will maximise drug-carrying capacity.

The primary goal of this study is to focus on developing a novel technology to protect bioactives from microbial degradation in the rumen and target their release to the abomasum. Such technology is a microcapsule that is closed in the rumen and open in the abomasum, using pH as the stimulant or switching gate to change microcapsule permeability. Three stages are involved:

1. Preparation – fabricating microcapsules using various polymers and three methods:
    - Interfacial polymerisation
    - Solvent evaporation technique
    - Phase inversion technique
2. Functionalisation – modifying the surface of microcapsules by two routes:
    - Plasma induced grafting
    - Chemical grafting
3. Characterisation – measuring physical properties of the functionalised microcapsules and evaluating with model drugs:
    - Stability
    - Strength
    - Size
    - Porosity
    - Release study

2.7.2 Criteria and considerations

Based on the literature review on controlled release mechanisms, together with the desire to have a smart system that operates when an environmental factor such as pH changes, several factors need to be researched to develop a successful method for targeted drug delivery. These are the basis of the research and are summarised in Figure 2.10.

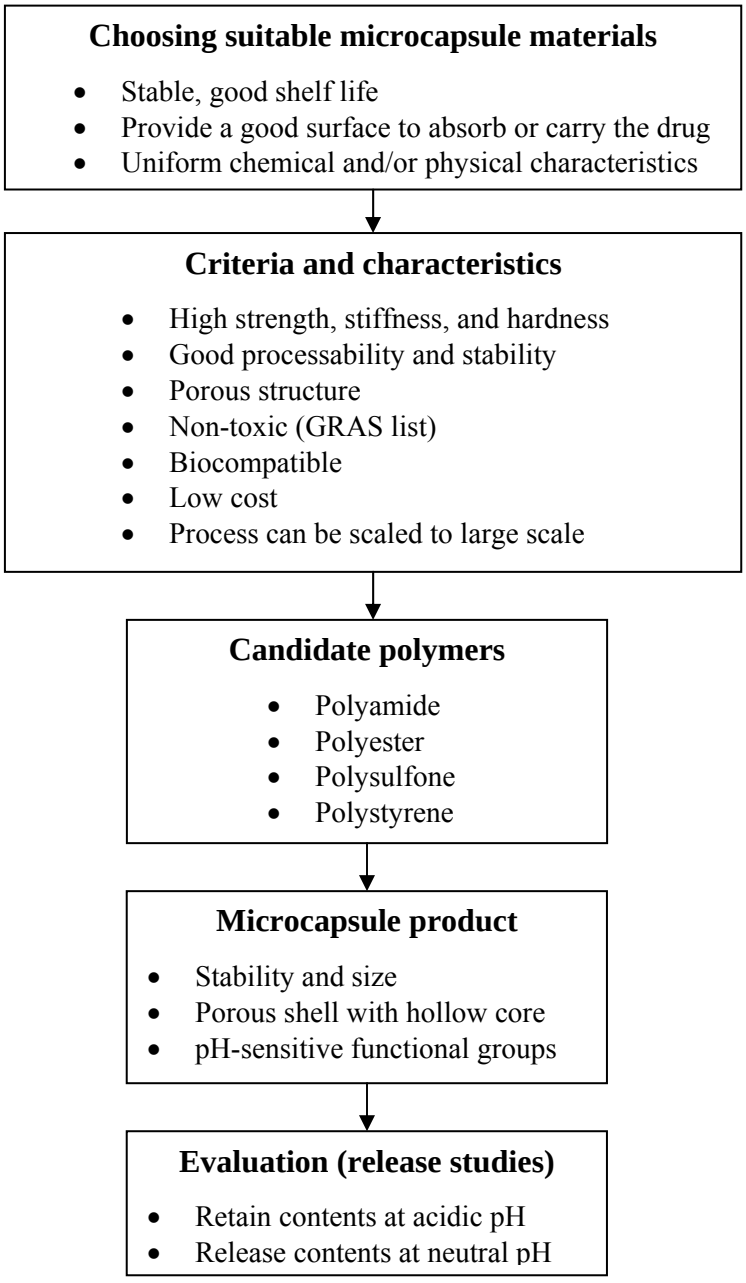

Figure 2.10 Criteria and considerations for the research